RESIDUE REVIEWS

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Residues of Pesticides and Other Contaminants in the Total Environment

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Foreword

Worldwide concern in scientific, industrial, and governmental communities over traces of toxic chemicals in foodstuffs and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published progress reports, and archival documentations. These three publications are integrated and scheduled to provide in international communication the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. Until now there has been no journal or other publication series reserved exclusively for the diversified literature on "toxic" chemicals in our foods, our feeds, our geographical surroundings, our domestic animals, our wildlife, and ourselves. Around the world immense efforts and many talents have been mobilized to technical and other evaluations of natures, locales, magnitudes, fates, and toxicology of the persisting residues of these chemicals loosed upon the world. Among the sequelae of this broad new emphasis has been an inescapable need for an articulated set of authoritative publications where one could expect to find the latest important world literature produced by this emerging area of science together with documentation of pertinent ancillary legislation.

The research director and the legislative or administrative advisor do not have the time even to scan the large number of technical publications that might contain articles important to current responsibility; these individuals need the background provided by detailed reviews plus an assured awareness of newly developing information, all with minimum time for literature searching. Similarly, the scientist assigned or attracted to a new problem has the requirements of gleaning all literature pertinent to his task, publishing quickly new developments or important new experimental details to inform others of findings that might alter their own efforts, and eventually publishing all his supporting data and conclusions for archival purposes.

The end result of this concern over these chores and responsibilities and with uniform, encompassing, and timely publication outlets in the field of environmental contamination and toxicology is the Springer-Verlag (Heidelberg and New York) triumvirate:

Residue Reviews (vol. 1 in 1962) for basically detailed review articles concerned with any aspects of residues of pesticides and other chemical contaminants in the total environment, including toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for *Residue Reviews* and the *Archives* are in identical formats and are subject to review, by workers in the field, for adequacy and value; manuscripts for the *Bulletin* are not reviewed and are published by photo-offset to provide the latest results without delay. The individual editors of these three publications comprise the Joint Coordinating Board of Editors with referral within the Board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

December 1, 1975 Coordinating Board of Editors

Preface

That residues of pesticide and other contaminants in the total environment are of concern to everyone everywhere is attested by the reception accorded previous volumes of "Residue Reviews" and by the gratifying enthusiasm, sincerity, and efforts shown by all the individuals from whom manuscripts have been solicited. Despite much propaganda to the contrary, there can never be any serious question that pest-control chemicals and food-additive chemicals are essential to adequate food production, manufacture, marketing, and storage, yet without continuing surveillance and intelligent control some of those that persist in our foodstuffs could at times conceivably endanger the public health. Ensuring safety-in-use of these many chemicals is a dynamic challenge, for established ones are continually being displaced by newly developed ones more acceptable to food technologists, pharmacologists, toxicologists, and changing pest-control requirements in progressive food-producing economies.

These matters are of genuine concern to increasing numbers of governmental agencies and legislative bodies around the world, for some of these chemicals have resulted in a few mishaps from improper use. Adequate safety-in-use evaluations of any of these chemicals persisting into our foodstuffs are not simple matters, and they incorporate the considered judgments of many individuals highly trained in a variety of complex biological, chemical, food technological, medical, pharmacological, and toxicological disciplines.

It is hoped that "Residue Reviews" will continue to serve as an integrating factor both in focusing attention upon those many residue matters requiring further attention and in collating for variously trained readers present knowledge in specific important areas of residue and related endeavors involved with other chemical contaminants in the total environment. The contents of this and previous volumes of "Residue Reviews" illustrate these objectives. Since manuscripts are published in the order in which they are received in final form, it may seem that some important aspects of residue analytical chemistry, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology are being neglected; to the contrary, these apparent omissions are recognized, and some pertinent manuscripts are in preparation. However, the field is so large and the interests in it are so varied that the editors and the Advisory Board earnestly solicit suggestions of topics and authors to help make this international book-series even more useful and informative.

"Residue Reviews" attempts to provide concise, critical reviews of timely advances, philosophy, and significant areas of accomplished or needed endeavor in the total field of residues of these and other foreign chemicals in any segment of the environment. These reviews are either general or specific, but properly they may lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology; certain affairs in the realm of food technology concerned specifically with pesticide and other food-additive problems are also appropriate subject matter. The justification for the preparation of any review for this book-series is that it deals with some aspect of the many real problems arising from the presence of any "foreign" chemicals in our surroundings. Thus, manuscripts may encompass those matters, in any country, which are involved in allowing pesticide and other plant-protecting chemicals to be used safely in producing, storing, and shipping crops. Added plant or animal pest-control chemicals or their metabolites that may persist into meat and other edible animal products (milk and milk products, eggs, etc.) are also residues and are within this scope. The so-called food additives (substances deliberately added to foods for flavor, odor, appearance, etc., as well as those inadvertently added during manufacture, packaging, distribution, storage, etc.) are also considered suitable review material. In addition, contaminant chemicals added in any manner to air, water, soil or plant or animal life are within this purview and these objectives.

Manuscripts are normally contributed by invitation but suggested topics are welcome. Preliminary communication with the editors is necessary before volunteered reviews are submitted in manuscript form.

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Table of Contents

DDT metabolism in microbial systems

By

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Contents

I. Introduction

The metabolic fate of DDT[1] is of interest not only because of widespread concern for environmental pollution but also because it affords an opportunity to study the complex metabolic reactions carried out in different organisms and various other ecosystems. Although DDT has been used for more than three decades, much of the knowledge of its metabolism in different systems is incomplete, misleading, or fraught with

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[1] Throughout this paper, DDT refers to *p,p'*-DDT. The chemical names and structures of this and other abbreviations used are listed in the Glossary.

inconsistencies. Only with the advent of gas-liquid (GLC) and thin-layer chromatography (TLC) and mass spectrometry (MS) coupled with more extensive use of radiolabeled compounds has real progress been made.

For many years, DDE and DDA were considered to be the only major metabolites from biological systems until microbial systems were studied (MATSUMURA and BOUSH 1971). Part of the reason for this was that the standard Schechter-Haller colorimetric method of analysis did not distinguish DDD (TDE) from DDT so it is not surprising that it was not until 1963 that TDE was shown to be formed from DDT in animal tissues (FINLEY and PILLMORE 1963). Until recent years, the persistence of DDT in natural ecosystems was attributed to microbial inability to degrade it and its related metabolites.

Although countless research papers and numerous reviews have been published on the metabolism of DDT and related compounds, no specific review on its metabolism in microbial systems has been published. In the past few years, however, a number of general reviews have appeared which include microbial metabolism of DDT to varying degrees of thoroughness (ALEXANDER 1972, BOLLAG 1972, FRIES 1972, MATSUMURA and BOUSH 1971, MEIKLE 1972, MENZIE 1969, PFISTER 1972).

Microorganisms have assumed an ever increasing importance in the study of pesticide degradation. Various environments or ecosystems supporting or capable of supporting large microbial populations (soil, water, sewage, etc.) have long been thought to be ideal sites for degradation but supporting data were meager. The finding that anaerobic conditions were necessary for noticeable degradation caused an upsurge in research efforts. Natural anaerobic environments are found in river and lake bottoms, in soils, and in the rumen and intestinal systems of many animals. In sludge digestion of municipal and industrial wastes, and in ensilage, similar anaerobic environments exist. Since these systems have undefined and usually very diverse microbial populations, much research effort has been directed toward using various isolates in attempts to find organisms capable of degrading DDT. For this reason, studies with undefined microbial populations will be reviewed separately from those involving defined populations. I have attempted to review the literature in this broad, multidisciplinary field, much of which has appeared since 1970, and to evaluate the work done by reference to the original literature. Every effort has been made to include the more pertinent and recent information to June 1974.

II. Nonbiological degradation and other considerations

There are numerous examples of pesticides, including DDT, undergoing transformations by nonmetabolic processes (CROSBY 1969 and 1970, KEARNEY and HELLING 1969). These processes include those initiated by light, water, pH, heat, free radicals, and the complex mixtures of organic and inorganic chemicals in soils. CASTRO (1964) has shown that reduced

iron porphyrin complexes are oxidized by DDT and TDE is formed. Similarly, MISKUS *et al.* (1965) observed the same dechlorination in aqueous solutions of reduced porphyrins under anaerobic conditions. GLASS (1972) reported that the iron redox system in water-saturated soil was capable of degrading DDT to TDE and the rate of TDE formation was related to the rate of ferrous iron formation and that this occurred also in a soil-free iron redox system. ECHOBICHON and SASCHENBRECKER (1967) questioned the concept of enzymatic dechlorination of DDT when they found DDT to be converted to TDE and DDE plus unknown metabolites in frozen heparinized blood that was repeatedly thawed. This suggested the involvement of iron porphyrins. However, the fact that they thawed the blood repeatedly would not only release enzymes for activity, but others may have been activated in the process. Overall, many of the papers reviewed herein present strong evidence that the observed degradations are enzymic. OTT and GUNTHER (1965) pointed out that various "metabolites" can be formed during analytical procedures, especially those involving GLC.

These findings indicate the importance and difficulty in ascertaining whether transformations are biological or are chemical. The work cited regarding porphyrins is especially difficult since reduced iron porphyrins are present in all aerobic organisms and are long-lived in the environment. In addition, KLEIN *et al.* (1964 and 1965) reported the isomeric conversion of *o,p'*-DDT to *p,p'*-DDT in the rat. Since these initial reports, it has been shown that this conversion does not take place (CRANMER 1972, BITMAN *et al.* 1971). The *p,p'*-DDT was shown to be an impurity in the *o,p'*-DDT which was detected due to the more rapid metabolism of *o,p'*-DDT which resulted in an elimination rate differential (BITMAN *et al.* 1971).

Although the simplest procedure for distinguishing between biological and nonmetabolic pesticide breakdown is in comparing the rate of decomposition in sterile and nonsterile systems, care must be exercised in the method of sterilization to prevent untoward changes (ALEXANDER 1965). Perhaps, as stated by CROSBY (1969), many of the *in vitro* studies of pesticide degradation involve both biological and nonbiological processes.

III. Undefined microbial populations

a) Soil

Reviews by ALEXANDER (1965) and EDWARDS (1966) point out that DDT is very stable in soils. Even though one of the primary functions of soil microorganisms is the decomposition of a wide array of organic compounds in soil, there are numerous examples of materials, both biological and synthetic, which persist from years to millenia (ALEXANDER 1972). The most abundant soil microorganisms are bacteria but, because of their small cell size, the fungi actually account for the greater portion of micro-

bial mass in most soils (ALEXANDER 1961). Culture studies of micro-organisms isolated from soil will be reviewed in later sections under their taxonomic grouping.

A number of studies of DDT degradation in soil have shown that under anaerobic conditions reductive dechlorination predominates, whereas under aerobic conditions dehydrochlorination is the dominant reaction. GUENZI and BEARD (1967) found ^{14}C-DDT (ring-labeled) converted directly to TDE when incubated in a moist soil in a CO_2-N_2 atmosphere. After four weeks, TDE accounted for 62% of the recovered radioactivity, 34% as DDT and 4% as other products. However, 43% of the radioactivity was not recovered and they found no activity in either a hexane or NaOH trap designed to trap volatilized materials. Only part of the missing radioactivity was in the water layer of the partitioned hexane extract and they did not try to isolate and identify water-soluble compounds. They indicated finding small amounts of DDA, DDE, BA (*p*-chlorobenzoic acid), dicofol, DBP, and DDM. Autoclaving of replicate soil samples for 1 hr prevented DDT degradation and the authors concluded that the degradation processes were of microbial origin. Shortly thereafter, GUENZI and BEARD (1968) compared DDT degradation under aerobic and similar anaerobic conditions with and without a 1% alfalfa amendment. They found that alfalfa enhanced the degradation of DDT under anaerobic, but not aerobic conditions, finding less than 1% as DDT after 12 weeks. Recovery decreased with time, even in sterile soil, and only 64% of the radioactivity added was recovered after 12 weeks of anaerobic incubation and only 46% was in identifiable compounds. Even combustion of residual carbon after solvent extraction and trapping of the CO_2 brought the total recovery to only 79%. Since in both anaerobic studies they flushed the incubation chambers with nitrogen only at the completion of incubation, the low recoveries, even with combustion, may indicate inadequate recoveries of residues residing in the chambers. This aspect was not described adequately; however, this latter study pointed out that alfalfa, as an added energy source, stimulates microbial activity which hastens the rate of DDT degradation. As GRAY (1970) pointed out, soil micro-organisms live under starvation conditions and are largely inactive. Therefore, the addition of an organic energy source should stimulate microbial growth.

KO and LOCKWOOD (1968) confirmed the work of GUENZI and BEARD but used water-logged soil. Using several amendments added at the 1% level, they found the conversion of DDT to TDE to be most effective with alfalfa, slightly less so with a peptone-glucose mixture, and least with barley straw. The amendments did not enhance degradation in aerobic soils. They found no conversion of DDT in sterile amended soil for up to five weeks indicating microbial involvement in degradation. They reported TDE to be more stable in soil than DDT and that TDE had a broader antimicrobial spectrum than DDT which may account for its longer soil persistence.

PARR *et al.* (1970 a) pointed out that laboratory studies using different means of obtaining anaerobic conditions may not be comparable because of varying effects on microorganisms. In a subsequent paper (1970 b), they confirmed the work cited above of enhanced DDT degradation to TDE in anaerobic soils amended with energy sources. They found DDT degradation after four weeks in moist soil to approach 100% in soil amended with alfalfa meal, rice straw, or hulls and 85% with glucose. Whereas recoveries of DDT and metabolites exceeded 95% after four weeks in moist aerobic soil, where degradation was minimal, recoveries were often only 60 to 80% in soils where extensive metabolism occurred. This was thought to be due to degradation of DDT to polar metabolites not extracted or detected.

JOHNSEN *et al.* (1971), in a related study with cattle manure as the amendment, showed that even after one week most of the DDT had disappeared from flooded soil. TDE was the major metabolite found with only traces of DDE found. Recoveries were uniformly low. Yet incubation in unamended moist soil resulted in recoveries exceeding 96% after one week. To check for other metabolites, 2 mg of DDT in 50 g of flooded soil amended with manure were incubated for one month resulting in a recovery of 71 percent. Only 63 μg of DDT was found, but 1,322 μg of TDE, 21 μg of DDMS, 12 μg of DDMU, 4 μg of DBP, and 3 μg of DDE were identified indicating a slow conversion of TDE to other products. An 18-day time-course study showed recoveries exceeding 90% through the first six days and thereafter decreasing. TDE increased in concentration through 12 days after which it decreased, indicative of the breakdown of TDE. No breakdown products of DDE or TDE were detected when these two compounds were incubated in flooded soil amended with manure. Since autoclaving can cause undesirable soil changes, they incubated 2 mg of DDT in manure-amended soil treated with 40 mg of $HgCl_2$. After one week they found no evidence of conversion to TDE, DDE, or other metabolites indicating that the metabolites found were of biological origin.

BURGE (1971) showed that the dechlorination of DDT to TDE was of biological origin by adding a small amount of viable soil to sterile soil which restored its ability to degrade DDT. He also found that oxygen atmospheres as low as 2% inhibited the dechlorination of DDT and recovery was quantitative. However, with nitrogen only 59% was recovered as DDT and TDE and 41% was unaccounted for after 64 days' incubation. No other metabolites, including DDA, were found. BURGE found both alfalfa and alfalfa-steam distillate to accelerate the anaerobic but not aerobic disappearance of DDT. With alfalfa distillate amendment, he found both TDE and DDE to be stable both aerobically and anaerobically in soil incubated for 31 days. He concluded that unrecovered DDT had not been converted to unidentified compounds through DDE or TDE as intermediates. However, the answer may not be that straightforward. Although direct information is lacking, DDT presumably is being

dechlorinated after it enters a microbial cell and the resulting TDE may then be further metabolized. According to MEIKLE (1972), the major barrier between a foreign organic compound and the metabolic machinery of a microorganism is the cytoplasmic membrane. Perhaps there is a membrane transport differential which DDT can cross to the exclusion of TDE. MEIKLE went into considerable detail on various aspects of membrane structures and membrane penetration rates which are germaine to these considerations. BURGE (1971), in other experiments, could not account for up to 74% of added DDT. CASTRO and YOSHIDA (1971) reported that TDE accumulated in four flooded, DDT-treated soils and fastest in the one with the highest organic matter content. They also showed that TDE was more persistent in these soils than DDT but residues diminished; they did not indicate finding any TDE metabolites.

In all these studies in which DDT disappeared in biologically active anaerobic soils with the accumulation of TDE, recoveries were less than ideal and the various authors speculated as to the cause. KEARNEY *et al.* (1966), using ^{14}C-DDT, found that up to 20% of the ^{14}C could not be extracted from flooded soils after a four-week incubation. They concluded that the ^{14}C-activity is tightly bound to soil particles in some form and that this loss was real and reproducible, although the mechanism was not known. These missing residues, however, may be locked into microbial matrices rather than soil particles.

b) Sewage

HILL and MCCARTY (1967) were the first to report the breakdown of DDT in sewage sludge. They incorporated DDT into a thick, biologically active, anaerobic, digested wastewater sludge and found DDT to be converted almost immediately into TDE. TDE in turn gradually was degraded with a half-life of about four days. Under aerobic conditions, with several milligrams of dissolved oxygen/L, DDT remained unchanged. They determined the degradation of TDE to follow first-order kinetics, but were unable to classify DDT due to its rapid conversion to TDE.

Further work with sewage sludge did not appear until late 1972. ALBONE *et al.* (1972 a), using DDT-treated anaerobic sewage sludge incubated under hydrogen, found by GLC analysis three peaks coinjecting with TDE, DDMS, and DBP. TDE was confirmed by GLC-MS and the peak corresponding to DBP was shown not to have arisen from dicofol. Papers by ALBONE *et al.* (1972 b) and JENSEN *et al.* (1972), appearing back to back in the same journal, reported a new metabolite of DDT from anaerobic sewage sludge abbreviated DDCN [bis (*p*-chlorophenyl) acetonitrile]. These are the first reports of a nitrogen-containing DDT metabolite. ALBONE and coworkers incubated both enriched and unenriched anaerobic sewage sludge with DDT for periods up to 88 days. Only a trace of DDT remained while TDE predominated and three

other GLC peaks relative to DDT of 0.37, 0.57, and 0.62 were found. The latter peak was identified as DDCN by combined GC-MS with synthetic DDCN exhibiting identical GLC and TLC characteristics. They found DDCN to represent 11.7% of the DDT initially incorporated. They speculated that DDCN could be formed from DDA through amide to nitrile formation or directly from DDT. JENSEN and coworkers found DDT to have a half-life of seven hours in their anaerobically incubated activated sludge. They also found DDCN in a local sewage sludge sample in Sweden, the first report of its occurrence in nature. They confirmed the identity of the DDCN using GLC-MS and chemical degradation. Since no DDCN could be detected after adding TDE or DDE to sludge, they postulated its direct formation from DDT. They found DDCN to represent 9% of the original ^{14}C-activity with an overall ^{14}C recovery of 40%. In neither paper did the authors ascertain whether DDCN was formed metabolically or chemically.

PFAENDER and ALEXANDER (1972) incubated ^{14}C-DDT in sewage for periods up to 24 weeks. TDE and DBP were the major metabolites accumulating but significant quantities of DDMU, DDMS, DDNU, DDM, and DBH also were found. Volatile organic compounds or $^{14}CO_2$ were not produced in significant amounts indicating little or no ring-cleavage occurred. Recovery increased with time and the authors speculated that this was possibly due to binding of DDT to organic matter and microbial cells which was released as the organic material was decomposed. Incubation of DBP in sewage collected at two different times showed DBP to disappear completely in four weeks from one sample and no degradation after six weeks in the other, indicative of seasonal microbial fluctuations. Similarly PCPA (*p*-chlorophenylacetic acid), found by FOCHT and ALEXANDER (1971) to be a ring-cleavage product of DDM, was incubated in sewage and found to disappear rapidly after the fourth week and to be essentially gone after six weeks. Sterilization by autoclaving prevented any loss of PCPA. Sterile controls were used throughout and any chemical changes were subtracted from the results reported, which in all cases were less than 5%. Since sterile air was passed over the sewage, strict anaerobiosis was not observed. In a subsequent study, PFAENDER and ALEXANDER (1973) incubated DDT in raw sewage with added inorganic salts. Glucose was added to four samples (two sterilized), diphenylmethane to another set, and a third set received no additions; the three sets were incubated for seven weeks. As in their 1972 paper, the same products were formed but the amounts varied with the amendment. TDE, DBP, and DDE represented 95% of the metabolites formed. By interval sampling, they found TDE to be formed at reasonably rapid rates in unamended sewage, markedly enhanced by glucose and reduced by diphenylmethane. DBP was formed slowly in the unamended sewage but both amendments reduced its formation even though both amendments resulted in marked bacterial population buildups with the effect of glucose being more rapid.

c) Sediment

In studies related to sediments, MATSUMURA *et al.* (1971) studied the metabolism of DDT by unidentified microbial isolates from top silt and bottom silt from Lake Michigan and its tributaries. Of the isolates tested, 77% from top silt (81 of 104) and bottom silt (72 of 92) formed TDE. For DDNS formation, 57 of 104 isolates from top silt and 41 of 92 from bottom silt were active. About one-third of the isolates also formed DDE. Incubation of ^{14}C-TDE with active isolates indicated that DDNS was formed by dechlorination of TDE (analogous to TDE formation from DDT). In TLC analyses, spots corresponding to TDE accompanied the DDNS spots. PATIL *et al.* (1972), in similar studies, investigated ^{14}C-DDT degradation in marine environments using unidentified microbial isolates from bottom sediments from bays, estuaries, and ocean floors. Sea bottom isolates were weak in degradative capacity but others formed TDE with lesser amounts of DDNS and DDOH. Varying amounts of unextracted activity, depending on the isolate, resided in the aqueous phase after extraction, due presumably to their polar nature. Their isolate no. 1708, for example, had 74% in the aqueous phase. Additional work on the identity of these compounds is needed.

Very few studies of DDT metabolism in sediments have appeared. ALBONE *et al.* (1972 a) studied DDT degradation in estuary sediments both *in situ* and *in vitro*. *In vitro* incubations produced greater conversion of DDT to TDE than *in situ* studies and was the only metabolite observed although small amounts of polar materials were evident from TLC plates. The search for $^{14}CO_2$ in the one experiment conducted was negative. JENSEN *et al.* (1972) found the new metabolite DDCN also in a lake sediment layer in Sweden at 0.6 ppm on a dry weight basis. PFAENDER and ALEXANDER (1972) also incubated ^{14}C-DDT in a fresh water-sediment ecosystem for periods up to 24 weeks. As with sewage, the major metabolites accumulating were TDE and DBP with small but significant amounts of DDE, DDMU, DDMS, DDNU, DDM, and DBH also being found. With both sewage and sediment, they reported 99.9% recovery of radioactivity using a 6 hr continuous extraction with diethyl ether, and also no evidence of ring-cleavage.

d) Silage

The first report of silage having an effect on DDT was by THORNBURG (1963) who mentioned briefly that corn silage degraded DDT to TDE. FRIES *et al.* (1969 a) ensiled alfalfa treated with DDE, TDE, and DDT *in vitro* for periods of 28, 56, and 84 days at room temperature. With recoveries of about 90%, they found only 20% of the DDT converted to TDE after 84 days, which is quite low in comparison to other studies cited. They found no change in DDE and did not report on TDE. HENZELL and LANCASTER (1969), in a more extensive study, used a rye grass and a rye grass mixture, which had been sprayed with technical

DDT, as silage. The technical material contained 75.8% *p,p'*-DDT, 23.8% *o,p'*-DDT, 0.4% DDE, and 0.015% DDMU. They incubated the silage for periods up to 90 days at 25 and 38°C and found the formation of *p,p'*-TDE and *o,p'*-TDE to parallel the level of their DDT isomers in the silage. The higher temperature resulted in a more rapid TDE formation and about 90% loss of DDT compared to 75% at 25°C. However, the formation of TDE from DDT resulted in a net loss of 50%; the remainder was unaccounted for.

e) Water

MISKUS *et al.* (1965) studied the conversion of ^{14}C-labelled DDT to TDE by six lake water samples incubated for seven days. They found that the extent of conversion was greater in samples with large amounts of plankton with a 95% conversion in one sample. No change in DDT was evident in either distilled water or boiled distilled water under vacuum indicating that the reaction was biological.

EICHELBERGER and LICHTENBERG (1971) evaluated the persistance of 28 pesticides in raw river water over a period of eight weeks. They found no measurable changes, either biological or chemical, nor any loss of either DDT, TDE, or DDE. Nothing is mentioned regarding the microbial life in the water but it is expected that there would be numerous bacteria and algae. It is surprising then, in light of work already cited and cited in other sections, that no conversion of DDT to TDE was found.

OLOFFS *et al.* (1972) treated water samples from two rivers and from a subtidal zone of Canada with DDT and incubated them in the laboratory for periods up to 12 weeks. They determined bacterial counts periodically with values generally of 10^4 bacteria/ml and yet found no evidence of degradation. Although they showed substantial loss of DDT with time, glass wool plugs in the flasks only partly accounted for the loss. It seems that their analytical technique, employing extraction in a separatory funnel, would be at fault since this would not readily remove residues from within bacterial cells. Such cells would likely reside in the aqueous phase which was discarded.

The first report of the metabolism of DDT by aquatic microorganisms in pure culture was made by MATSUMURA *et al.* (1971). They tested the metabolic ability of unidentified microbial isolates from the water of Lake Michigan and three tributaries to degrade DDT. They found a large majority of 109 isolates capable of forming TDE (90 of 109). A considerable number of the isolates also produced DDNS (48). In testing the degradation of TDE the finding of DDNS implies that DDNS formation from TDE was the chief metabolic pathway. They postulated that DDT is dechlorinated to TDE which in turn is dechlorinated to DDNS. They also found that 22% of the isolates also formed DDE. In studies related to that above, PATIL *et al.* (1972) investigated the metabolic transformations of ^{14}C-DDT by microorganisms in marine waters off Hawaii and off